SEROLOGICAL INVESTIGATION OF DROSOPHILA ANTIGENS USING THE PRECIPITATION REACTION

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INTRODUCTION

The purpose of this investigation was to determine whether the precipitation reaction can be used in differentiating antigens of various *Drosophila* species. In an earlier study the complement fixation reaction was employed (Cumley and Haberman). The antigen-antibody relationships, determined by the two methods, were not found to correspond exactly when computed on a percentage basis. The order of reactivity of a series of antigens to a given antiserum, as determined by the two technics, was found to be much the same. The data presented in either of these preliminary reports do not warrant a final conclusion regarding the antigenic relationships existing between the species of *Drosophila* investigated.

MATERIALS AND METHODS

Since, at present, there is no information regarding the specific fractions of the *Drosophila* antigens, no attempt was made in either this investigation or in the previous one to remove fractions soluble in ether, alcohol, or chloroform. The saline extracts, standardized in terms of their nitrogen contents, were employed as antigens. This method may find objectors, since the antigenic components of certain bacteria are known to be alcohol soluble. On the other hand, a highly specific alcohol-insoluble substance has been isolated from moth tissue (Martin & Cotner, 1934). Several competent workers have insisted that the precipitin test is lipoid sensitive, and that the lipoids must be removed from the antigen before accurate specific differences can be obtained (Becker, 1932; Boyden, 1936; Moritz, 1934). Hence, there was no means of anticipating which fractions of the *Drosophila* contained the antigenic principles. Immunization of the rabbits has been reported in the previous experiments.

The "ring test" of Ascoli was applied in the manner used by Fornet and Muller (1910), because it gave easily readable results and required small quantities of reagents. Several minor modifications were made in order to adapt the test to the problem in hand. The precipitin tubes were 6 mm. in outside diameter and 40 mm. in length. Capillary pipettes were used for layering the antigen over the antiserum. In the tests about 0.2 cc. of undiluted serum was placed in each of ten tubes by means of capillary pipettes. Ten dilutions of antigen were prepared, varying from 1:50 to 1:1200. These dilutions were made from the original saline extracts that had been standardized in terms of nitrogen content. The original extracts were themselves 1:50, and consequently for the first tube, they were not diluted. About 0.2 cc. of each antigen dilution was layered over the antiserum in each tube. Care was taken to prevent mixing of the two reagents at the serum-antigen interface. Serum-saline and antigen-saline controls were set up and incubated with the tests at 37 degrees Centigrade. The tests were read after one and three hours. The formation of a precipitate at the interface indicated a positive test. The tubes in the dilution series were read for amounts of precipitation and recorded in terms of plusses. The strongest ring reactions were indicated by four plusses (++++), the weakest by one plus (+), and the negative reactions by a minus (−).

RESULTS

Readings taken after one hour of incubation revealed definite rings of precipitation, but titres were low and irregular upon retesting. After three hours, higher and more regular titres resulted, and these were the readings which are presented in this report. Occasionally, by the end of three hours, the precipitate had begun to diffuse throughout the serum. End-points of reactivity occurred in antigen dilutions of from 1:50 to 1:1200.

In Table 1 are shown the results of tests in which a given antiserum is tested against its homologous antigen and several heterologous antigens. The *Drosophila* species are arranged in the order of the relative reactivity of their antigens to a given antiserum. Two antigens that are adjacent in any given test are not necessarily similar. Their respective positions are merely indicative of the

TABLE 1

Type of Antiserum	Type of Antigen	Dilution of Antigen									
		1: 50	1: 75	1: 100	1: 150	1: 200	1: 300	1: 400	1: 600	1: 800	1: 1200
Mulleri 3	Mulleri	++++	++++	++++	++++	++++	++	+	+	+	+
	Virilis	++++	++++	++++	++++	++	+	+	+	–	–
	Hydei	++++	++++	+++	++	+	+	+	+	+	–
	Caribbea	++	++	+	+	+	+	+	–	–	–
	Bipectinata	++	++	+	+	+	–	–	–	–	–
	Melanogaster	+	+	–	–	–	–	–	–	–	–
Mulleri B5	Mulleri	++++	++++	++++	+++	+++	++	++	+	+	–
	Virilis	+++	+++	+++	+++	+++	++	++	–	–	–
	Hydei	+++	+++	+++	++	+	–	–	–	–	–
	Caribbea	+	+	+	–	–	–	–	–	–	–
	Bipectinata	++	+	–	–	–	–	–	–	–	–
	Melanogaster	–	–	–	–	–	–	–	–	–	–
Virilis A 4	Virilis	++++	++++	++++	++++	+++	+++	++	++	+	–
	Mulleri	++++	++++	++++	++	+	+	+	+	–	–
	Caribbea	+++	+++	+++	++	++	+	+	–	–	–
	Hydei	+++	+++	+++	++	++	+	–	–	–	–
	Bipectinata	–	+	+	–	–	–	–	–	–	–
	Melanogaster	–	–	–	–	–	–	–	–	–	–

TABLE 1—(*Continued*)

Type of Antiserum	Type of Antigen	Dilution of Antigen									
		1: 50	1: 75	1: 100	1: 150	1: 200	1: 300	1: 400	1: 600	1: 800	1: 1200
Caribbea C 1	Caribbea	+++	+++	++	++	++	++	+	+	–	–
	Hydei	++	++	++	++	+	–	–	–	–	–
	Bipectinata	+++	+++	+	+	+	–	–	–	–	–
	Virilis	+++	+++	++	+	–	–	–	–	–	–
	Melanogaster	+++	+++	+	+	–	–	–	–	–	–
	Mulleri	++	++	+	–	–	–	–	–	–	–
Caribbea C 4	Caribbea	++++	++++	++++	++++	+++	+++	++	+	+	+
	Hydei	++	++	++	+	+	+	+	+	–	–
	Melanogaster	+++	+++	++	++	+	+	+	–	–	–
	Virilis	++	++	++	+	+	+	–	–	–	–
	Bipectinata	++++	+++	+++	++	+	–	–	–	–	–
	Mulleri	++	+	+	–	–	–	–	–	–	–
Melanogaster B1	Melanogaster	++++	++++	++++	+++	++	++	++	+	–	–
	Caribbea	++++	++++	++++	+++	+++	+	+	–	–	–
	Hydei	+++	++	++	+	+	+	–	–	–	–
	Mulleri	–	–	–	–	–	–	–	–	–	–
	Virilis	–	–	–	–	–	–	–	–	–	–
	Bipectinata	–	–	–	–	–	–	–	–	–	–

reactivity of each to the serum in question. As in the case of the complement fixation reactions, there is considerable variability in the antisera of the rabbits immunized to the same antigen.

Table 2 presents the antigen-antibody relationships that have been calculated from the data of Table 1. The values were calculated on the basis of the highest dilution of reactivity as suggested by Boyden (1926, 1932, 1934), and on the basis of the total number of plusses, *i.e.*, the strength of reactivity, as suggested by Nelson and Birkeland (1929). These computations reveal the degree of reactivity between a given antigen and antibody, when compared with the homologous antigen-antibody reaction. The value of this work in the differentiation of species specific substances cannot be postulated at the present time.

TABLE 2

Type of Antiserum	Type of Antigen	Percentage Antigen-Antiserum Relationship: highest dilution of reaction (Boyden)	Percentage Antigen-Antiserum Relationship: total number of plusses (Nelson & Birkeland)
Mulleri	Mulleri	100.0	100.0
	Virilis	50.0	77.1
	Hydei	45.8	57.1
	Caribbea	22.9	22.4
	Bipectinata	13.1	18.8
	Melanogaster	Less than 6.3	Less than 7.2
Virilis	Virilis	100.0	100.0
	Mulleri	75.0	66.6
	Caribbea	50.0	55.6
	Hydei	37.5	51.8
	Bipectinata	12.5	7.4
	Melanogaster	.....	.....
Caribbea	Caribbea	100.0	100.0
	Hydei	41.7	48.5
	Melanogaster	29.2	49.1
	Bipectinata	25.0	52.2
	Virilis	25.0	44.8
	Mulleri	12.5	23.0
Melanogaster	Melanogaster	100.0	100.0
	Caribbea	66.6	91.0
	Hydei	50.0	59.1
	Mulleri	.....	.....
	Bipectinata	.....	.....
	Virilis	.....	.....

By comparing the data in Table 2 with those in Table 7* of the preceding complement fixation investigation, one may readily see that the antigen-antibody relationships, determined by the two methods do not correspond. The percentage relationship between any antigen and antibody is never quite the same in the two tables. The order of relationship however, of any series of antigens to a given antiserum is quite similar. Several authors have reported discrepancies of this sort, and the view was once held that two different antigen-antibody complexes were involved (Topley and Wilson, 1936, p. 166). More recently, however, these apparent inconsistencies have been attributed to the fact that the complement fixation and precipitation reactions are really the secondary results of a single antigen-antibody reaction, but that these results are obtained under different physical conditions. In the present case, either of these two causes may have given rise to the lack of agreement between the two tests. However, it is more likely that experimental error and inaccuracies of standardization will account for these discrepancies.

Exact reciprocal antigen-antibody relationships were not obtained, as may be seen in the data of Table 2. The same condition was observed in the complement fixation study. Although the suggestion has been made that reciprocal relations should exist between any two species (Boyden, 1934), there is considerable evidence that such a law is not universal. In the bacteria, results similar to those presented here have been obtained. There is a multiplicity of antigenic fractions in bacteria, and any sharing of these components will result in some reciprocal relationships. These shared components, however, are not always present in the same proportions in each of the species tested (Wilson and Miles, 1932). Thus it is that "a given dose of bacteria containing the antigens A and B in the proportions 3a plus 2b might remove all agglutinins from a serum containing the corresponding antibodies in the proportions 3A plus 2B; but the same dose of bacteria containing the same antigens in the proportions 2a plus 3b, while more than sufficient to remove all the B agglutinins, would leave some of the A agglutinins unabsorbed" (Topley, 1935, pp. 92–93). This condition would possibly account for the fact that several

* Jour. N. Y. Ent. Soc., vol. XLVI, no. 4, p. 412, Dec., 1938.

workers, using as antigens helminths (Eisenbrandt, 1936), wheats (Nelson & Birkeland, 1929), and molluscs (Makino, 1934) have failed to get strictly reciprocal values. Likewise, a sharing of antigens in unequal proportions is probably the reason for the lack of reciprocal antigen-antibody relationships among the *Drosophila* species.

CONCLUSION

The precipitation reaction has been used to show differences in the antigens of various *Drosophila* species. The values obtained by the precipitin test did not correspond exactly with those obtained by the complement fixation reaction, probably due to factors inherent in the two different technics or to errors in performing the tests. Reciprocal antigen-antibody relationships may or may not be found, depending upon whether the antigenic components of the various species are shared in equal proportions.

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DR. WALTHER HORN

The many friends of Dr. Horn, in this country, will be saddened by the news of his death which occurred at Berlin-Dahlem, on July 10, 1939, after much suffering. Dr. Horn was in his 68th year. He was cremated at Berlin-Wilmersdorf on July 15.